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Polybrominated diphenyl ethers and metabolites – An analytical review on seafood occurrence

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ABSTRACT

Environmental health is systematically compromised by persistent toxic substances, which may have serious implications in terms of food safety issues and, thus, in general public health. In this context, polybrominated diphenyl ethers (PBDEs) and their biologically active metabolites have been increasingly assessed in seafood, the main route of human exposure.

As a consequence, a multiplicity of solvent-assisted analytical approaches is now available to accurately determine tiny amounts of these contaminants in complex matrices, like seafood. However, the majority of analytical procedures lead to high organic solvent consumption, thereby also contributing to the deterioration of environmental health.

The current review provides up-to-date information and critical discussion regarding the most common methodologies applied in the determination of PBDEs and their metabolites in seafood (2006–2016), from sample preparation to instrumental analysis. The ultimate goals of this comprehensive survey are to sensitize field researchers to work under the principles of green chemistry and to improve the global consciousness on the potential necessity of their regulation in foodstuffs.

1. Introduction

BFRs are chemical substances that are added to a wide variety of industrial and household products to reduce their flammability. They can be incorporated as either additives (mechanically blended with the polymeric material) or reactive ingredients (covalently attached to the polymer) [1]. Therefore, additive flame retardants may leach more easily to the environment than the reactive ones [1].

Nowadays, due to their reduced cost and recognized high efficiency and stability, PBDEs are often found in plastics, textiles and electrical/electronic equipment [1]. However, owing to environmental disposal of waste containing these additive flame retardants, PBDEs now have a widespread existence in the environment, even in locations far from where they were produced or used [2]. Moreover, due to their non-polar character, these compounds can easily suffer bioaccumulation and biomagnification, reaching all trophic levels [3–5]. Also, their toxicity in human and wildlife organisms, associated mostly with endocrine disruption [6], raised an increased legal and health concerns worldwide. In fact, many countries (e.g., European Union countries, The United Stated of America, China and Canada) have already some of these PBDEs under strict legislation (including Stockholm Convention) and surveillance in terms of their manufacture and further use in everyday life products [7–13].

Abbreviations: ACS, American Chemical Society; APCI, atmospheric pressure chemical ionization; BFR, brominated flame retardant; CCG, chemically converted graphene; CRM, certified reference material; CSE, conventional Soxhlet extraction; d-SPE, dispersive solid-phase extraction; ECD, electron capture detector; ECNI, electron capture negative ion; EFSA, European Food Safety Authority; EI, electron ionization; ESI, electrospray ionization; GC, gas chromatography; GCB, graphitised carbon black; GPC, gel permeation chromatography; HR, high resolution; IDL, instrumental detection limit; IS, ion spray; K_{OA}, octanol–air partition coefficient; K_{OW}, octanol–water partition coefficient; LC, liquid chromatography; LMR, laboratory material reference; LoD, limit of detection; MAE, microwave-assisted extraction; MeO, methoxylated; MS, mass spectrometry; MSPD, matrix solid-phase dispersion; NIST, National Institute of Standards and Technology; OH, hydroxylated; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; P_L, vapour pressure; PLE, pressurized liquid extraction; PSA, primary secondary amine; PTV, programmed temperature vaporizer; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; RfD, reference doses; SFE, supercritical fluid extraction; SIM, selected ion monitoring; SPE, solid-phase extraction; SPME, solid-phase microextraction; UAE, ultrasound-assisted extraction; WW, wet weight.

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Additionally, recent evidences of biotransformation of PBDEs, resulting in MeO-PBDEs and OH-PBDEs metabolites with identical toxicological repercussions [14], indicate that the risk of exposure to endocrine disruptors may be even greater than previously thought. Due to their structural resemblance with PBDEs, they are also environmentally persistent and have been found in various seafood species.

PBDEs comprise several brominated neutral aromatic compounds with a chemical structure consisting of two phenyl rings linked by an ether bond (Fig. 1A). The PBDE family embraces theoretically 209 congeners, sharing the same substitution pattern and congener numbering system of system of PCBs [15]. Unlike their precursors, neither OH-PBDEs nor MeO-PBDEs are intentionally synthesized or used for industrial purposes. Many hundreds of theoretical structural metabolites of PBDEs may exist under the chemical structure represented in Fig. 1B. However, analogues with a 2,4-dibromo substitution pattern (relative to the diphenyl ether bond) in the non-hydroxy-/non-methoxy-containing ring, as well as the hydroxy-/methoxy-substituted *ortho* to the diphenyl ether bond substitution, are the most frequently detected in the environment and biota [16]. Consequently, some of these PBDE metabolites have been frequently called as "naturally produced PBDEs" [3,16,17]. However, there is not enough solid evidence to support these claims, though radiocarbon studies to ascertain if the origin of these compounds is anthropogenic or natural would be of major relevance [17,18].

Current understanding assumes that non-occupational human exposure to such persistent organic pollutants relies mainly on a combination of exposure through diet, ingestion/inhalation of indoor dust and inhalation of indoor air [6]. Among these, the dietary route plays a considerable role on the overall human exposure, especially from contaminated seafood consumption [19], but there are no admissible limits for the presence of PBDEs and their metabolites in food so far. Even so, in 2008, the U.S. Environmental Protection Agency has derived RfDs (i.e. an estimate of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects to humans during a lifetime) for four PBDEs, for its Integrated Risk Information System [20]. RfD for PBDEs 47, 99, 153, and 209, were defined as 0.1, 0.1, 0.2 and 7 mg/kg/day, respectively [20].

Throughout the past decades, owing to the recognized value of seafood as a part of a healthy diet, there has been a remarkable promotion of seafood consumption [21]. Therefore, due to its significant role to human exposure, for the past fifteen years, approximately three hundred scientific articles have been published on PBDEs presence in fishery products (based on ECsafe-SEAFOOD online database, www.ecsafeseafooddbase.eu) [22]. Unfortunately, the majority of studies on PBDEs and metabolites are focused on method development with a small number of seafood samples instead of full monitoring studies to support environmental impact assessments. From all the studies previously revised by Cruz and co-workers [23], the highest levels of total PBDEs have been detected in benthic or benthopelagic species as common carp (12,700 μg/kg, WW basis) or burbot (19,970 μg/kg, WW basis) [24,25]. The most frequently quantified congeners in seafood are BDE-47, -99, -100, -153, -154, -183 and -209 (Fig. 2), all included in the list of those whose production is already under regulation [9]. This fact might be related to their likely presence in the PentaBDE (e.g. DE-71, Bromkal 70-5DE) and DecaBDE (e.g. Saytex® 102E, Bromkal 82-0DE) technical mixtures extensively used in many countries until their restriction or banishment. MeO-PBDEs and OH-PBDEs amounts are much more variable between studies, though the congeners found in higher concentrations are a result of both BDE-47 and -99 metabolism (Fig. 2) [26]. The authors also confirmed that total MeO-BDEs amounts reached up to 11.60 μg/kg (on a dry weight basis) in mackerel [26]. Despite OH-PBDEs are often found in lower amounts than MeO-PBDEs, substantial levels (up to 3450 μg/kg, WW basis) were verified in blue mussels [27]. Generally, the proportion of each class varies considerably between specimens, though PBDEs are present in higher amounts (80% of total PBDE-like compounds), followed by MeO-PBDEs (15%) and OH-PBDEs (5%) in seafood samples.

Being present in residual levels, a wide range of extraction and cleanup techniques, together with many instrumental approaches, have been developed and employed for their identification and quantification in seafood products. This analytical variety hardens the set-up of official guidelines to enable their assessment and,

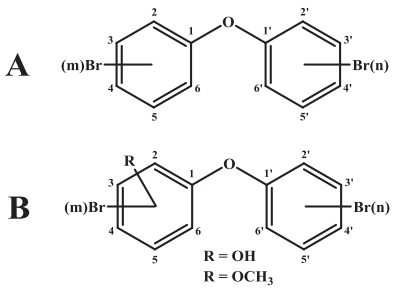

Fig. 1. The general chemical structure of (A) polybrominated diphenyl ethers [Br(m,n) = 0–5, m + n ≥ 1] and (B) hydroxylated (R = OH) and methoxylated (R = OCH_3) polybrominated diphenyl ethers [Br(m,n) = 0–5, m + n ≥ 1].

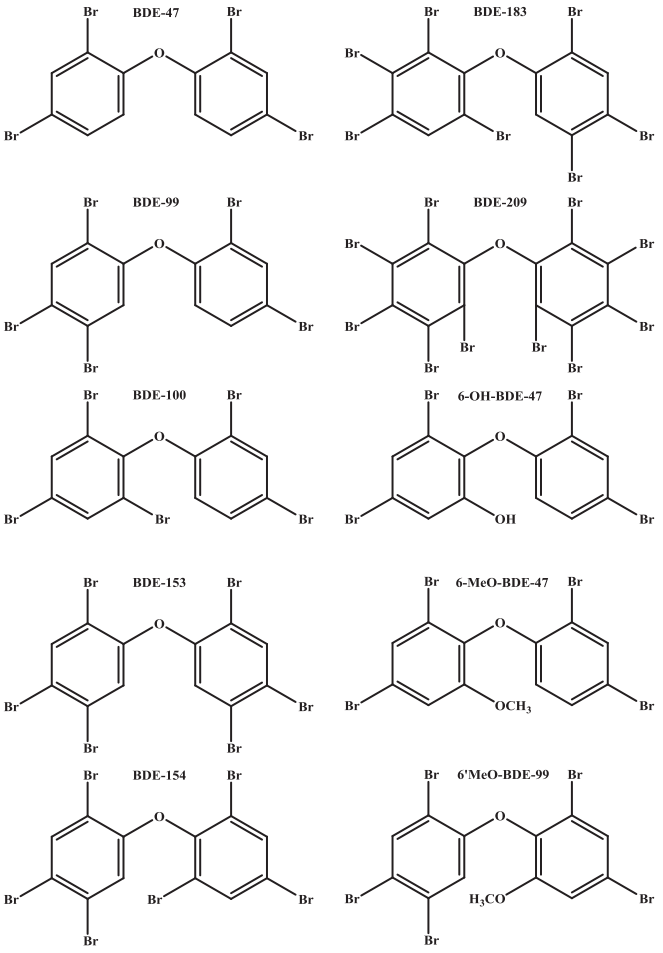

Fig. 2. Chemical structures of most prevalent congeners of polybrominated diphenyl ethers and metabolites.

consequently, to define regulatory limits on their presence in food. In addition, common analytical procedures seem to be also a cause of additional damage to the environment, mostly owing to high organic solvent consumption.

In this way, it is necessary to develop reliable tools to quantify these chemicals in food products and evaluate their real toxicity, in order to sensitize and support the authorities and food industry to implement legal control measures on chemical hazards within food safety assurance schemes. For this purpose, the present review provides a critical and organized overview of the most common analytical procedures for the determination of PBDEs and their metabolites in seafood, while emphasizing potential improvements that environmental-friendly analytical approaches may bring to these assessments.

2. Physicochemical characterization

2.1. Polybrominated diphenyl ethers

In order to understand the behaviour of a specific compound in nature, namely its environmental persistency and wide spreading potential or elimination, several physicochemical features need to be taken into consideration, particularly in a huge and complex family of compounds as PBDEs. Similarly, when attempting to develop an analytical method for the assessment of a certain substance, in particular one that is present in extremely low amounts in a complex matrix, knowledge of their physicochemical features is mandatory, especially for extraction and cleanup steps.

PBDEs molecular weights vary from 249 (for mono-BDEs) to 959 (for deca-BDE) with typical ^{79}Br (50.5%) and ^{81}Br (49.5%) isotope distribution patterns [28].

Hence, depending on bromination degree, ten PBDE classes may be pointed out: mono-BDEs (BDE-1, -2 and -3), di-BDEs (BDE-4 to BDE-15), tri-BDEs (BDE-16 to BDE-39), tetra-BDEs (BDE-40 to BDE-81), penta-BDEs (BDE-82 to BDE-127), hexa-BDEs (BDE-128 to BDE-169), hepta-BDEs (BDE-170 to BDE-193), octa-BDEs (BDE-194 to BDE-205), nona-BDEs (BDE-206, -207 and -208) and deca-BDE (also referred to as BDE-209). Furthermore, PBDEs exist mostly in twist or skew conformations, and the higher the degree of bromination in *ortho* position is, the more skewed the conformation [29].

Despite being relevant physicochemical parameters, information regarding measured melting and boiling points, instead of calculations, is scarce. Admire and colleagues [30] were able to verify a melting point variation from 19°C (BDE-3) to 308°C (BDE-209) and a boiling point fluctuation from 306°C (BDE-3) to 411°C (BDE-47). No data on congeners with a high degree of bromination (more than four bromine substituents) was found.

Water solubility is a key feature that affects the transformation of a compound in water through hydrolysis, photolysis, oxidation, reduction, and biodegradation reactions [31]. Regarding PBDEs, all classes show extremely low aqueous solubility (BDE-15 = 0.821 mol/L; BDE-209 = 0.002 mol/L), which is strongly correlated to their molecular mass and molecular volume [31]. Due to such reduced water solubility, water stability assays are nearly impossible to execute for some PBDEs (as BDE-209).

The K_{OW} is imperative in determining the environmental fate of lipophilic organic chemicals, particularly in biota. In fact, PBDEs are highly hydrophobic, since their log K_{OW} may achieve 8.27 in BDE-183, being tri- and tetra-BDEs those within the range of optimum bioaccumulation potential [32].

K_{OA} is also used to describe chemicals' mobility in the atmospheric environment and can be estimated from K_{OW} and Henry's law constant [33]. For PBDEs, log K_{OA} may vary from 9.30 (BDE-17) to 11.97 (BDE-156), at 25°C, based on the assessed compounds, being also affected by molecular volume and degree of bromination [33]. Therefore, these pollutants would not be easily transported via atmosphere. Since log K_{OA} is temperature-dependent, so it is PBDEs surface-air partition [33]. In fact, partition into octanol increases by an average factor of 3 for every 10°C decrease in temperature [33]. Therefore, the lower the atmospheric temperature is, the smaller the PBDE fraction in air. This fact helps to explain their presence in remote locations where no production sites exist, recognizing PBDEs ability to long-range atmospheric transport as a result of a series of deposition/volatilization events, known as the "grasshopper effect", which is also enhanced by vegetation air–surface exchange [34].

Another crucial property in terms of physicochemical characterization is P_L, which allows predicting behaviour of organic micro contaminants in the environment and calculating Henry's law constants [31]. Despite vapour pressure may vary according to the experimental method used for its assessment, the increase of molecular volume and number of substituted bromine at the *ortho* position results in a linear reduction of a certain PBDE vapour pressure [31]. Hence, log P_L may range from around −0.7 (BDE-1) to −6.2 (BDE-190), thus corroborating their low volatility [31].

2.2. Metabolites

Similar to their precursors, OH-PBDEs and MeO-PBDEs show a high bioaccumulative and biomagnificative potential due to their high log K_{OW}. For instance, among the tested MeO-PBDEs, 6-MeO-BDE-17 presented the lowest lipophilicity (log K_{OW} = 5.74) and 6-MeO-BDE-138, the highest (log K_{OW} = 7.67) [35]. In addition, from the analysed OH-PBDEs, 6-OH-BDE-138 was the most hydrophobic (log K_{OW} = 7.17), and 2-OH-BDE-7, the least (log K_{OW} = 4.63) [35].

No data was found regarding log P_L of OH-PBDEs or MeO-PBDEs, though log K_{OA} varied from 8.30 (2′-OH-BDE-3) to 13.29 (6-OH-BDE-157), for HO-BDEs, and from 10.16 (2′-MeO-BDE-28) to 13.00 (6-MeO-BDE-157), for MeO-PBDEs [36].

While PBDEs and MeO-PBDEs are known as neutral compounds, OH-PBDEs present a predictive pK_a that range from 4.2 to 9.3 [37], depending on the substitution pattern (*ortho*-, *meta*-or *para*-substitution) of both bromine and hydroxyl substitutes and bromination degree. Hence, during sample extraction/cleanup, if the matrix is not acidified to a pH below 4, then extraction of all OH-PBDEs may not be fully accomplished [37].

3. Environmental degradation

Current knowledge indicates that the major PBDEs degradation products or metabolites are less-brominated PBDEs, OH-PBDEs and MeO-PBDEs. Due to their strong hydrophobicity and low solubility in water, PBDEs reveal high partition affinity to organic matrices/solvents. In general, higher brominated BDEs degrade faster than less-brominated ones in apolar solvents, though different degradation rates occur within the same bromine number PBDE class [38]. Furthermore, PBDEs photoreactivity order is generally *meta* ≥ *ortho* > *para*, particularly for PBDEs with less than eight bromines [38]. Also, photoreactivity of PBDEs is lower in poor hydrogen-donating solvents, as tetrahydrofuran, being debromination the main mechanism of PBDEs photolysis in organic solvents [38]. On the other hand, in natural aquatic environment, photochemical behaviour depends strongly on concomitant chemical species, such as humic substances, metal and halide ions, often generating OH-PBDEs, chlorinated PBDEs, besides the less-brominated PBDEs [38]. In the atmosphere, photochemical transformation of PBDEs can be described as direct photolysis and photooxidation by OH radicals, thus resulting in the formation of OH-PBDEs [38].

4. Sampling and sample preparation

Food matrices are known to be highly heterogeneous, being composed by water, carbohydrates, fats, proteins, and minerals, which also count with a potential diversity of micronutrients, additives and contaminants. This complexity may be even further increased after food thermal processing. As a consequence, food samples are inherently difficult to analyse accurately.

From an analytical point of view, seafood is amongst the most complex food products, not only due to its chemical composition but also owing to what is considered as being edible (e.g. muscle, muscle plus skin or whole body with or without viscera, all depending on the species under study and dietary habits). Additionally, the size of the food portion to be analysed, as well as the seafood tissues/organs represent key points in the overall analytical process, especially when attempting to estimate the dietary intake of a certain nutrient or contaminant.

In order to obtain a representative and homogeneous laboratory sample, without secondary contamination, EFSA advises the analysts to proceed according to the provisions set out for dioxins and dioxin-like PCBs in food [39]. This regulation provides a number of requirements regarding sampling methods for seafood and other goods, defining precautionary measures during sampling, the size of the lot, packaging, transport, storage, sealing and labelling. From therein, some key points may be highlighted as collecting fish of comparable size and weight, i.e. differences do not exceed 50% [39]. Moreover, collection of incremental samples may vary according to fish size [39]. Still, when sampling fish muscle, care should be taken that no epidermis or subcutaneous fatty tissue are included in the sample [28]. If liver dissection is intended, care should be taken to avoid contamination from other organs [28]. If bile samples are to be taken, they should be collected first [28].

Regarding shellfish sampling, a 24 h depuration may be required to void the gut contents of any associated contaminants before freezing or sample preparation, depending on the purpose of the study [28]. Shellfish should be shucked, live if possible, and opened with minimal tissue damage, by detaching the adductor muscles from the interior of at least one valve [28].

No sampling or sample preparation recommendations were found for other types of seafood, as cephalopods or crustaceans. Nevertheless, selection of target species should also attend some empiric criteria as potential bioaccumulation capacity, geographic distribution, easier identification as possible, abundance, easy capture, distinct trophic levels and habitats, and adequate size to provide acceptable tissue for analysis. Also, considering the increasing presence of PBDEs and active metabolites, seafood consumption patterns should also be considered.

4.1. Pre-extraction determinations

Aiming to characterize samples and report their concentrations in biota on a dry-weight or lipid-weight basis, moisture and lipid contents are usually evaluated prior to extraction. Dry weight should be determined gravimetrically, while the total lipid content of seafood is often determined using CSE. Meanwhile, extracted lipids may be further used for analysis, if protective measures are taken during extraction. Otherwise, the lipid content should be determined on a separate subsample of the tissue homogenate. Seafood samples must then be frozen, at −20°C or lower, freeze-dried (optional) and protected from light until analysis [28].

5. Extraction and cleanup

While sample preparation steps remain nearly identical (e.g., sample fraction collection, freezing, freeze-drying), extraction and cleanup procedures have been moving towards time- and cost-effective multi-residue analysis with the lowest possible LoD. However, the majority of available analytical methodologies are still far from being fully considered as environmental-friendly, especially due to the high solvent consumption.

The concept of “Green Chemistry” was firstly defined by Anastas and Warner as the design of chemical products and processes that minimize or eliminate the generation of harmful substances to the environment and humans [40]. The first principle of green chemistry, defined as “Prevention”, brings awareness on the prevention of waste, rather than its posterior treatment or cleaning [40]. Therefore, the use of solventless extraction techniques, application of direct determination methodologies, and miniaturization are envisaged. However, for chromatographic determinations, the former procedures can only be applied to relatively clean matrices, as otherwise the chromatographic columns would easily deteriorate due to deposition of non-eluted sample components. Therefore, although molecularly-imprinted polymers have shown good outcomes for abiotic matrices [41], the use of solventless extraction and cleanup methods for PBDEs analysis in seafood is still far from optimized.

5.1. Extraction methods

The most frequently used techniques for the extraction of PBDEs and metabolites in seafood are presented in Fig. 3, which gathers data from 140 published scientific papers in ACS, Elsevier, Taylor & Francis and Wiley, regarding seafood contamination by PBDEs, from 2006 to 2016. The keywords used for this research were: “polybrominated diphenyl ether”, “PBDE”, “fish”, and “seafood”.

It is very clear that, in 2006, CSE was the most popular extraction method for PBDE and metabolites analysis in seafood (Fig. 3). Nevertheless, there has been a well-defined trend in using more environmental-friendly methods with PLE and UAE gaining ground over the most conventional ones. Other extraction methods, as highlighted in Fig. 3 are QuEChERS approaches, MSPD, MAE and SFE.

5.1.1. Conventional Soxhlet Extraction

This technique consists of an exhaustive thermal extraction of organic analytes by an apolar solvent, semi-continuously refluxed through the sample, in a Soxhlet system. This cycle may be allowed to repeat over a few hours or even days.

Regarding PBDEs and derivatives in seafood, CSE usually lasts 21 h, on average (Table 1), but it may reach 72 h of extraction [42].

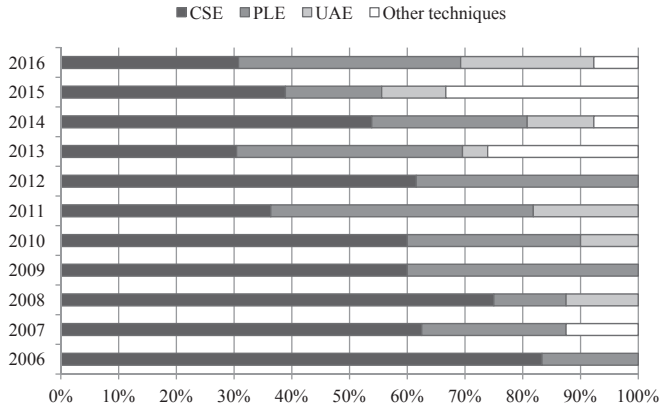

Fig. 3. Time-trend (2006–2016) representation of the extraction techniques most used in polybrominated diphenyl ethers analysis in seafood. CSE, Conventional Soxhlet Extraction; PLE, Pressurized Liquid Extraction; UAE, Ultrasound-Assisted Extraction.

Table 1
Summary of selected reported studies from the past decade regarding CSE of PBDEs and their metabolites in seafood.

Solvent	Ratio	Extractive volume (mL)	Sample (g)	Time (h)	Congeners	Matrix	Ref.
HEX:DCM	1:1	180	4, DW	24	17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190	Muscle, liver and eggs	[47]
		200	3, DW	48	Total PBDEs (Σ9)	Muscle	[66]
		–	20, WW	16	15, 17,28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190, 203, 204, 205, 206, 207, 209	Muscle	[92]
	5:1	120	3–5, DW	20	28, 47, 99, 100, 153, 154, 183	Muscle	[93]
HEX: ACT	1:1	400	5, DW	72	Total PBDEs (–)	Muscle	[42]
		200	12, WW	48	Total PBDEs (Σ7)	Muscle	[54]
	3:1	100	10, WW	2	Total PBDEs (Σ7)	Muscle and whole body	[83]
	4:1	350	–	7	Total PBDEs (Σ11)	Muscle and whole body (zooplankton)	[84]
		–	10, DW	16	28, 47, 99, 100, 153, 154, 183; 5-MeO-47, 6-MeO-47, 2′-MeO-68	Muscle	[94]
DCM: ACT	1:1	80	5, DW	12	15, 17, 28, 47, 66, 99, 100, 153, 154, 183, 209	Muscle and liver	[95]
		–	1.5, DW	18	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191	Muscle	[46]
		100	2, DW (muscle); 1, WW (liver)	24	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209; 5-MeO-47, 6-MeO-47, 4-MeO-49, 2-MeO-68, 5-MeO-99, 5-MeO-100, 4-MeO-101, 4-MeO-103	Muscle and liver	[96]
Diethyl ether: HEX	–	–	15–30, DW	7–8	Total PBDEs (Σ14)	Whole body	[55]
TOL	–	–	40, WW	20	28, 47, 88, 99, 153, 183	Muscle and whole body	[97]
DCM	–	–	1–3, DW	12	47, 66, 85, 99, 100, 153, 154, 183	Muscle, liver, spleen, stomach, stomach content, blood, egg, gallbladder	[98]

ACT, acetone; CSE, Conventional Soxhlet Extraction; DCM, dichloromethane; DW, Dry Weight; HEX, n-hexane; PBDEs, PolyBrominated Diphenyl Ethers; TOL, toluene; WW, Wet Weight.

Despite the fact that Soxhlet system dimension may vary, the common procedures often use 80–400 mL of organic solvent to extract PBDEs and their derivatives from 3 to 5 g (on a dry weight basis) of fish muscle tissue (Table 1). Since these pollutants present very high log K_{OW}, lipid-rich matrices are more prone to present higher level of contamination. This implies that, when contaminants with high log K_{OW} are analysed in samples with high lipid content, a lower sample intake is required for quantification [43]. Therefore, the amount of sample used for analysis heavily relies on the matrix fat content, which is applied for all extraction methods.

Together with log K_{OW}, knowledge of the relative solubility of target analytes in various solvents can form the extraction and cleanup method basis. Binary solvent mixtures normally containing n-hexane:dichloromethane and n-hexane:acetone, in different proportions (often 1:1, 3:1 and 4:1, v/v), with an average volume of 165 mL per sample, are usually preferred for CSE, but single solvent extraction with toluene or dichloromethane is also observed (Table 1).

Despite CSE advantages, as operative availability, robustness and simplicity, this technique leads to high solvent and time consumptions that are not compatible to the green chemistry demands. In addition, the final extracts require further cleanup steps prior to instrumental analysis.

5.1.2. Pressurized Liquid Extraction

Aiming to overcome some CSE limitations, PLE (or Automated Soxhlet Extraction, ASE™, traded by Dionex Corporation) brought major green chemistry improvements. PLE is used for extraction of solid samples by using a liquid-phase at high pressure and/or temperature, but below its critical state. Briefly, the efficiency of extraction depends on the solvent/sample ratio, sample composition, particle size (preferably below 0.4 mm) and extraction time, as was with CSE [44].

Sample size and extraction solvents employed in PBDEs and metabolites analysis are usually the same as the ones used in CSE, where n-hexane:dichlorometane (1:1, v/v) or single dichloromethane stand out (Table 2). Aware of the known dichloromethane toxicity, despite being lower than that of chloroform used in the most classical lipid extraction methods in seafood (Bligh and Dyer or Folch methods), it is remarkable that this halogenated solvent is still so frequently used in "environmental-friendly" methods. However, since the extraction is carried out in closed-vessels under pressure (7–12 MPa) and at high temperatures (90–150°C), approaching a supercritical state, it can be faster (6–60 min) and use less organic solvent (50–100 mL, depending on solvent flow rate and number of cycles) than CSE (Table 2).

PLE systems may be used in static or flow-through modes. Despite being less efficient than the latter, static mode is often preferred by analysts to avoid extract dilution and reduce solvent consumption [44]. Other advantages of PLE are its autonomy (several samples can be processed sequentially), different sample sizes or extraction volume can be accommodated (e.g. 11–100 mL vessels are available, though 22 mL are more commonly chosen, Table 2).

Although its use is becoming more frequent (Fig. 3), PLE apparatus is still very expensive, less robust than CSE and extracts also require subsequent cleanup. Nevertheless, PLE selectivity may be increased by loading sorbents in the outlet end of sample cell, so undesired substances may be retained by the sorbent, thus providing an in-cell cleanup.

5.1.3. Ultrasound-Assisted Extraction

UAE is the simplest solid–liquid (or liquid–liquid, depending on sample nature) extraction method, which is carried out by blending the solid sample with an appropriate organic solvent and promoting "cavitation", using an ultrasonic bath or a closed extractor fitted with a sonic probe, the latter leading to more reproducible results.

The "cavitation" process consists on the formation and implosion of vacuum micro bubbles through the solvent, creating hot-spots with high temperatures and pressures (estimated up to 4726°C and 100 MPa), thus accelerating chemical reactivity of the medium [44]. As a consequence, the solvent penetrates more easily into solid materials and a mass transfer from inside out the matrix cells occurs, therefore enhancing extraction efficiency.

Table 2
Summary of selected reported studies from the past decade regarding PLE of PBDEs and their metabolites in seafood.

Solvent	Ratio	Sample (g)	Time (h)	Other features	Congeners	Matrix type	Ref.
HEX:DCM	1:1	–	0.3	Static mode, 100 mL vessel, T = 125°C, P = 10 MPa	47, 55, 99, 100, 153	Muscle, whole soft tissue and whole body (zooplankton)	[5]
		1, DW	0.85	Static mode, 22 mL vessel, T = 100°C, P = 10 MPa	28, 47, 49, 66, 99, 100, 119, 140, 153, 154, 183, 209	Muscle	[65]
		1, DW	–	Static mode, T = 100°C, P = 13.7 MPa	28, 47, 99, 100, 153, 154, 183	Muscle	[81]
		5, DW	0.1	Static mode, T = 110°C, P = 10 MPa	28, 47, 99, 100, 153, 154, 183, 209	Muscle and whole soft tissue	[99]
		2, DW	–	12 mL vessel, T = 90°C	17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 197, 201, 202, 203, 206, 207, 208, 209	Whole body	[67]
		2, DW	–	T = 150°C, P = 10 MPa	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183	Muscle	[58]
DCM	–	0.5, DW	0.25	Static mode, 22 mL vessel, T = 100°C, P = 13 MPa	Total PBDEs (Σ4)	Muscle	[63]
		_	0.33	T = 100°C, P = 6.9 MPa	Total PBDEs (Σ11)	Muscle	[100]
				Static mode, 22 mL vessel, T = 100°C, P = 13.8 MPa	28, 30, 33, 47, 75, 85, 99, 153, 154, 155; 6-MeO-47, 2′-MeO-68	Muscle	[101]
HEX: ACT	–	–	0.25	Static mode, T = 120°C, P = 10 MPa	28, 47, 66, 71, 77, 85, 99, 100, 119, 138, 153, 154, 183, 190	Muscle	[64]
	4:1	5–10, WW	0.35	Static mode, T = 100°C, P = 10.3 MPa	1, 2, 3, 7, 8, 10, 11, 12, 13, 15, 17, 25, 28, 30, 32 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190; 2′-MeO-28, 4'- MeO-17, 6-MeO-47, 6′-MeO-66, 2′-MeO-74, 2′-MeO-75; 4′-OH-17, 6-OH-47, 6′-OH-66, 2′-OH -74, 2′-OH-75	Whole body	[86]
HEX	–	25–50, DW	0.7	T = 125°C, P = 10 MPa	28, 47, 29, 66, 99, 100, 153, 154, 183	Muscle, liver, gonads and adipose tissue	[102]
ETN: TOL	1:5	–	–	–	28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, 209	Muscle and/or bone	[103]

ACT, acetone; DCM, dichloromethane; DW, Dry Weight; ETN, ethanol; HEX, n-hexane; PBDEs, PolyBrominated Diphenyl Ethers; PLE, Pressurized Liquid Extraction; TOL, toluene; WW, Wet Weight.

In general, the extraction efficiency is dependent upon ultrasound frequency, temperature, sonication time, extractive solvent and matrix nature, as well as sample particle size [44].

A pronounced advantage in comparison to the previous methods is that UAE allows extraction of large amounts of sample with a relatively low cost, usually being as fast as PLE (less than 1 h) and requires lower solvent volumes (5–90 mL, Table 3). However, it does not allow automation and the extractive solvents are commonly the same as those used in CSE and PLE.

5.1.4. Other extraction methods

Another extraction method that has been demonstrated to be an interesting alternative for extraction of PBDEs is MAE. This technique employs non-ionizing radiation (0.3–300 GHz) that causes molecular motion by ionic conduction and rotation of dipoles in both solvent and sample, resulting in thermal energy generation [44]. If a certain molecule presents relatively high dielectric constant, then the oscillation in the microwave field is more intense, causing dipole rotation and disruption of weak hydrogen bonds. Furthermore, polar solvents with high dielectric losses, such as water, are more efficient in producing heat, and so samples are usually dried prior to extraction to avoid overheating. On the contrary, non-polar solvents as n-hexane will not produce thermal energy. Therefore, it is of common practice the use of a binary mixture (e.g., n-hexane:dichloromethane 1:1, v/v) where only one of the solvents absorbs microwaves. Moreover, the higher the viscosity of the medium is, the lower the molecular rotation [44].

MAE may be performed in closed vessels under pressure (pressurized MAE) or in open vessels at atmospheric pressure (focused MAE). Although the former is less safe in terms of sample handling, it provides enhanced extraction speed and efficiency, being the most used MAE system in contaminants analysis. Briefly, the efficiency of extraction depends on the solvent (nature and solvent/sample ratio), temperature and pressure, extraction time, power, sample composition (moisture) and particle size (preferably 0.1–2 mm) [44]. This extractive technique also presents a reduced extraction time (around 15 min per batch of as many as 40 samples) and uses small amounts of solvents (40 mL in MAE, Table 4). However, the equipment is considerably expensive, the extract must be filtered after extraction, and further cleanup steps are generally needed.

While for other methods pressure control is not a crucial step (as its role is just to keep the extractive solvent in its liquid state), for SFE it is fundamental. This method implies the use of an extractive gas in its supercritical state, usually carbon dioxide (at around 31°C and 7.4 MPa), which results in a fluid that has higher diffusivity, thus it presents a higher solvating power and may extract analytes faster and more efficiently than liquids, providing solvent-free extracts. Briefly, extraction yield depends on solvent flow rate, sample composition, particle size, solubility (pressure and temperature balance) and the use of polarity modifiers [44].

Indeed, SFE provides highly selective extractions, by altering the density of the extractive fluid through pressure and temperature adjustments, resulting in cleaner extracts than those obtained by PLE, where co-extraction of matrix lipids is nearly inevitable. When intended, extraction selectivity may be further improved by using polar modifiers (e.g. ethanol, acetone or methanol), thus increasing the solvating power and reducing the analyte–matrix interactions [44]. Similarly to CSE and PLE, water removal from sample matrix is advised, since it negatively affects extraction [44].

Despite being a more environmental-friendly technique, SFE is still extremely expensive, reducing its widespread use and no papers on this matter were found during the period 2006–2016. Notwithstanding, in 2005, Rodil and co-workers [45] developed a SFE-based multiresidue analytical methodology that was combined with SPME for the assessment of several halogenated contaminants, including PBDEs, in seafood.

Finally, regardless of the method chosen for contaminants extraction, addition of internal standards should be done at this stage of the analytical procedure.

Table 3
Summary of selected reported studies from the past decade regarding UAE of PBDEs and their metabolites in seafood.

Solvent	Ratio	Extractive volume (mL)	Sample (g)	Time (h)	Congeners	Matrix type	Ref.
HEX:DCM	1:1	90	5–15, WW	1	17, 28, 47, 49, 66, 85, 99, 100, 101, 118, 126, 153, 154, 155, 183	Muscle, whole soft tissue and whole body (small fishes)	[53]
		40	3, DW	0.85	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 194, 195, 196, 201, 202, 205, 206, 207, 208, 209	Muscle	[57]
		30	1, DW	0.17	3′-OH-7, 3′-OH-28, 4′-OH-17, 2′-OH-28, 3-OH-47, 5-OH-47, 4′-OH-49, 6-OH-47	Muscle	[51]
	4:1	8	1, WW	0.5	47, 99, 100, 153	Muscle	[60]
HEX:Methyl tert-butyl ether	1:1	5	1, DW	0.5	28, 47, 66, 68, 85, 99, 138, 153, 154, 183; 2′-MeO-68, 6-MeO-47, 3-MeO-47, 5-MeO-47, 4′-MeO-49, 4-MeO-42, 6′-MeO-99, 5′-MeO-99, 6-MeO-85; 3′-OH-28, 4-OH-42, 3-OH-47, 4′-OH-49, 5-OH-47, 6-OH-47, 2′-OH-68, 6-OH-85, 5-OH-99, 6′-OH-99	Muscle and whole soft tissue	[69]
ACT:cHEX:water → ACT:cHEX	3:4:2 → 1:2	60	–	–	28, 47, 99, 100, 153, 154	Liver	[25]
cHEX:ACT	3:2	–	4–7, WW	–	Total PBDEs (Σ10)	Muscle	[76]
ACN:TOL	9:1	5	2.5, DW	–	28, 47, 99, 100, 153, 154, 183, 209	Muscle and whole soft tissue	[68]
ACN	–	5	5, WW	0.9	28, 47, 99, 100, 153, 154, 183	Fish liver and gonads	[104]

ACN, acetonitrile; ACT, acetone; cHEX, cyclohexane; DCM, dichloromethane; DW, Dry Weight; n-hexane; PBDEs, PolyBrominated Diphenyl Ethers; TOL, toluene; UAE, Ultrasound-Assisted Extraction; WW, Wet Weight.

Table 4
Summary of selected reported studies from the past decade regarding MAE and QuEChERS of PBDEs in seafood.

Method	Solvent	Ratio	Extractive volume (mL)	Sample (g)	Time (h)	Other features	Congeners	Matrix type	Ref.
MAE	HEX:DCM	1:1	40	0.25, WW	0.25	T = 115°C	Total PBDEs (–)	Muscle	[105]
	Nitric acid: hydrochloric acid	4:1	15	1–2, WW	–	–	Total PBDEs (Σ8)	Muscle	[56]
QuEChERs	ACN	–	10	10, WW	–	–	Total PBDEs (Σ7)	Muscle	[106]
				10, WW	–	–	28, 99, 100, 153, 154	Muscle	[107]
			4	4, DW	–	–	28, 47, 99, 100, 153, 154	Muscle	[108]
			5	5, WW	0.9	–	28, 47, 99, 100, 153, 154, 183	Fish liver and gonads	[104]
	Ethyl acetate	–	10	10, WW	0.12	–	28, 37, 47, 49, 66, 77, 85, 99, 100, 153, 154, 183, 196, 197, 203, 206, 207, 209	Fish muscle	[109]

ACN, acetonitrile; DCM, dichloromethane; DW, Dry Weight; HEX, n-hexane; MAE, Microwave-Assisted Extraction; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; PBDEs, PolyBrominated Diphenyl Ethers; TOL, toluene; WW, Wet Weight.

5.2. Cleanup methods

Even after selecting and optimizing the extraction procedure, several matrix components (e.g. lipids) or compounds with physicochemical similarities with our analyte might still remain in the extract. They must be removed to ensure a more accurate identification and sensitive quantification of PBDEs and metabolites, as well as to minimize deterioration of instrumental performance. The cleanup methods used for the analysis of this type of contaminants are often categorized as destructive (e.g. strong acid or alkaline treatment) or non-destructive methods for lipid removal (e.g. GPC or SPE).

5.2.1. Sulphur removal

Sulphur should be removed from sample extracts, to minimize interferences and to protect the detectors. This can be easily accomplished by adding copper powder during (and sometimes after) CSE or PLE, but it can also be removed by GPC. Sediment, soil and sewage sludge often contain significant amounts of this element, but the same does not happen for seafood. Yet, it is occasionally used as a precaution measure in the cleanup of seafood extracts [5,46,47].

5.2.2. Acid and alkaline treatments

Since PBDEs and MeO-PBDEs are stable under strong acid conditions, sulphuric acid or hydrochloric acid are frequent destructive treatments used to ensure an efficient removal of lipid matter. Lipid destruction in acidic conditions is commonly performed by adding acid directly to the sample extract (dissolved in an organic solvent) [48–50], which clearly leads to several liquid–liquid extractions and centrifugation steps, turning it into a laborious and long-standing approach. However, as previously highlighted (see section "Physicochemical Characterization), adequate recovery of OH-PBDEs can only be assured if acidification of sample extract is conducted prior to cleanup [37,51].

For seafood, other destructive methods for lipid removal, such as saponification with alcoholic alkalis (sodium or potassium hydroxide), have also been investigated [4,48], but it may result in losses of bromine atoms from highly brominated PBDEs [52].

5.2.3. Gel permeation chromatography

This method, also known as size exclusion chromatography, is based on molecular size separation and is mainly used to remove lipid material (greater than 500 Å) from sample extracts. When applied to the analysis of PBDEs and their derivatives in seafood, GPC often use Bio-Beads S-X3 (200–400 mesh, 2000 Da limit) gels in polystyrene-divinylbenzene columns and dichloromethane-based mixtures as eluent (Table 5).

In general, single GPC is sufficiently effective in isolating target compounds from co-extracted lipids (less than 5% lipid content) [53]. However, more complex matrices may require the

Table 5
Summary of selected reported studies from the past decade on cleanup methods for PBDEs and their metabolites analysis in seafood.

Analytical method	Organic solvents (mL)[a]	Other features (top to bottom)	Matrix type	Fat content (%)	Congeners	Recovery (%)	Ref.
CSE – **SPE**	HEX:DCM 7:3 (50)	H_2SO_4 → anh. Na_2SO_4 → activated copper → activated silica	Muscle	0.01–29.1	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191	76–108	[46]
	HEX:DCM 1:9 (50)	H_2SO_4 → anh. Na_2SO_4 → Florisil	Muscle	4.6–6.3	28, 47, 99, 100, 153, 154, 183; 5-MeO-47, 6-MeO-47, 2′-MeO-68	46–90	[94]
	HEX (30), HEX:DCM 7:3 (30)	H_2SO_4 → anh. Na_2SO_4 → acid silica → activated silica → Na_2SO_4 → acid alumina	Muscle and liver	–	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209; 5-MeO-47, 6-MeO-47, 4-MeO-49, 2-MeO-68, 5-MeO-99, 5-MeO-100, 4-MeO-101, 4-MeO-103	73–117	[96]
CSE – **d-SPE** – **SPE**	HEX (100), HEX:DCM 1:1 (150)	Acid silica gel → anh. Na_2SO_4 → florisil → acid silica → silica	Muscle	2.1	28, 47, 99, 100, 153, 154, 183	74–105	[93]
PLE – **SPE**	HEX (120), HEX:DCM 1:1 (60)	anh. Na_2SO_4 → deactivated Florisil → anh. Na_2SO_4 → acid silica → anh. Na_2SO_4	Whole body	3.8–6.1	17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 197, 201, 202, 203, 206, 207, 208, 209	57–116	[67]
	HEX (70), HEX:DCM 3:2 (30)	Acid silica → activated neutral alumina	Muscle	2.1–5.4	28, 47, 99, 100, 153, 154, 183	58–123	[81]
	–	anh. Na_2SO_4 → silica gel → acid silica → silica → basic silica → silica → anh. Na_2SO_4 → basic alumina → anh. Na_2SO_4 → carbon	Muscle	1.2–2.8	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183	75–127	[58]
PLE – **GPC** – **SPE**	HEX:DCM 1:5 (20)	Silica	Muscle	–	28, 30, 33, 47, 75, 85, 99, 153, 154, 155; 6-MeO-47, 2′-MeO-68	42–101	[101]
	HEX:DCM 1:1 (610), DCM (20)	Biobeads S-X3 → florisil	Whole body	3.9–8.6	1, 2, 3, 7, 8, 10, 11, 12, 13, 15, 17, 25, 28, 30, 32 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190; 2′-MeO-28, 4′-MeO-17, 6-MeO-47, 6′-MeO-66, 2′-MeO-74, 2′-MeO-75; 4′-OH-17, 6-OH-47, 6′-OH-66, 2′-OH -74, 2′-OH-75	23–106	[86]
MAE – **SPE**	HEX (300), HEX:DCM 3:2 (400)	Acid silica → anh. Na_2SO_4 → acid silica → basic silica → anh. Na_2SO_4 → silanised glass wool	Muscle	–	Total PBDEs (Σ8)	50–100	[56]
UAE – **SPE**	DCM (90), HEX:DCM 5:1 (60), HEX:DCM 97:3 (50)	Acid silica → anh. Na_2SO_4 → anh. Na_2SO_4 → Deactivated silica	Muscle and whole soft tissue	–	28, 47, 66, 68, 85, 99, 138, 153, 154, 183; 2′-MeO-68, 6-MeO-47, 3-MeO-47, 5-MeO-47, 4′-MeO-49, 4-MeO-42, 6′-MeO-99, 5′-MeO-99, 6-MeO-85; 3′-OH-28, 4-OH-42, 3-OH-47, 4′-OH-49, 5-OH-47, 6-OH-47, 2′-OH-68, 6-OH-85, 5-OH-99, 6′-OH-99	73–109	[69]
	–	H_2SO_4 → hydromatrix	Muscle	–	3′-OH-7, 3′-OH-28, 4′-OH-17, 2′-OH-28, 3-OH-47, 5-OH-47, 4′-OH-49, 6-OH-47	–	[51]
UAE – **SPE** – **d-SPE** – **SPE**	HEX (34), Ethyl acetate (8), ACN (5), HEX:DCM 1:1 (36)	Florisil → acid silica → C_{18}, Z-Sep → aminopropyl silica	Muscle and whole soft tissue	0.1–6.2	28, 47, 99, 100, 153, 154, 183, 209	78–144	[68]
MSPD – UAE – **SPE**	HEX (41), HEX:DCM 4:1 (35)	H_2SO_4 → acid silica → activated silica → neutral activated alumina	Muscle	3.2–15.2	3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 194, 195, 196, 201, 202, 205, 206, 207, 208, 209	56–119	[57]
MSPD	HEX:DCM 1:1 (0.5), Acetone (1)	Graphene → Florisil → anh. Na_2SO_4	–		17, 28, 47, 66, 85; 3′-MeO-28, 4-MeO-42, 3-MeO-47, 5-MeO-47, 6-MeO-47, 4′-MeO-49, 2′-MeO-68, 6-MeO-85, 5′-MeO-99, 6′-MeO-99; 3′-OH-28, 4-OH-42, 3-OH-47, 5-OH-47, 6-OH-47, 4′OH-49, 2′-OH-68, 6-OH-85, 5′-OH-99, 6′-OH-99	23.8–109.9	[72]
QuEChERs – **SPE**	Ethyl acetate (10), HEX (1), isooctane (0.5)	Silica	Muscle	1.6–14	28, 37, 47, 49, 66, 77, 85, 99, 100, 153, 154, 183, 196, 197, 203, 206, 207, 209	77–107	[109]

UAE – QuEChERs – **d-SPE**	ACN (5)	$MgSO_4$, primary-secondary amine, C_{18}	Liver and gonads	–	28, 47, 99, 100, 153, 154, 183	60–107	[104]
QuEChERs – **d-SPE**	ACN (4)	$MgSO_4$, primary-secondary amine, Z-Sep, C_{18}	Muscle	3–10	28, 47, 99, 100, 153, 154	85–119	[108]
LLE – **SPE**	HEX:DCM 3:1 (21), HEX:Acetone 85:15 (6), DCM:Methanol 88:12 (10), HEX (14)	anh. Na_2SO_4 → Florisil → H_2SO_4 → acid silica → neutral silica	Plasma	0.64–1.61	47, 99, 100, 153, 154, 183; 2′-MeO-28, 4′-MeO17, 2′-MeO-68, 6- MeO-47, 4′-MeO-103; 2′-OH-28, 4′-OH-17, 2′-OH-68, 6-OH-47, 5-OH-47, 4′-OH-49, 4-OH-42, 4′-OH-101	41–81	[49]
SLE – **SPE**	HEX (120), HEX:DCM 9:1 (100)	anh. Na_2SO_4 → 10% $AgNO_3$-silica → silica → acid silica → neutral silica → basic silica	Muscle, liver intestine	1.27–18.3	3, 7, 15, 17, 27, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, and 209; 3′-MeO-28, 5-MeO-47, 6-MeO-47, 4-MeO-49, 2-MeO-68, 5′-MeO-99, 5-MeO-100, 4′-MeO-101, 4-MeO-103; 3′-OH-28, 6-OH-47, 6-OH-100	38–125	[4]
SLE – **GPC** – LLE – **SPE**	HEX:DCM 1:1 (–), HEX:DCM 88:12 (–)	Biobeads S-X3 → neutral silica	Muscle and whole soft tissue	–	2′-OH-68 2′-MeO-68; 2′,6-diOH-68, 2′-OH-6-MeO-68, 2′,6-diMeO-68	76–93	[79]

ACN, acetonitrile; anh., anhydrous; DCM, dichloromethane; d-SPE, dispersive-Solid Phase Extraction; GPC, Gel Permeation Chromatography; HEX, n-hexane; LLE, Liquid–Liquid Extraction; MAE, Microwave-Assisted Extraction; MSPD, Matrix Solid-Phase Dispersion; PBDEs, PolyBrominated Diphenyl Ethers; PLE, Pressurized Liquid Extraction; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; SLE, Solid–Liquid Extraction; SPE, Solid-Phase Extraction; UAE, Ultrasound-Assisted Extraction.

[a] Organic solvent used in cleanup techniques highlighted (bold) in the preceding column.

employment of two serially connected GPC columns or GPC followed by further cleanup by SPE to achieve a suitable separation of PBDEs and metabolites from residual lipids and other organohalogenated compounds [5,53–55]. Despite not being able to remove all lipid traces, GPC presents itself as non-destructive and allows handling larger masses of lipids than SPE, while enabling its reuse, which is not possible with SPE.

5.2.4. Solid-Phase Extraction

The principle of SPE involves a partitioning of solutes between a liquid (sample matrix or extract) and a solid (sorbent) phase. This classic technique is used in an "off-line" mode and usually starts with sorbent conditioning, followed by sample application, subsequent rinsing and cleaning, and finally desorption and recovery of the analytes to be separated. Nowadays, SPE is more usually regarded as an extraction procedure. Nonetheless, regarding PBDEs and other pollutants, it is often employed as a cleanup step.

Considering sorbent technology, due to the lipid-soluble character of PBDEs and their metabolites, normal-phase inorganic based sorbents, as silica gel, alumina, Florisil® and silica chemically modified by polar groups such as amino, cyano or diol groups, are the most commonly utilized (Table 5). Generally, the use of acidified silica is enough to provide clean extracts, though several studies have described the use of acidified silica in combination with neutral silica and/or base-modified silica in multi-layer columns for enhanced purification [56–58]. For instance, 5 g of acidified silica (40%, w/w) is sufficient to purify 0.7 g of fish lipid samples for PBDEs analysis [59].

SPE usually constitutes a non-destructive (when acid or alkaline treatments are not employed), safe, efficient and reproducible technique. When compared to liquid–liquid extractions, SPE presents numerous advantages, since the former are usually more time-consuming (few hours per sample) and laborious (up to seven extraction steps) [4,48]. Moreover, a strict control of extraction conditions (e.g. temperature, pH and ionic strength) is required. Hence, SPE shows up as an attractive technique as it considerably provides more autonomy and, at the same time, high recoveries (often greater than 70%, Table 5).

Despite that some studies used miniaturized SPE [60], the majority still requires a huge amount of organic solvents to deliver clean extracts. In fact, around 200 mL of eluent is used to clean one sample extract, though it may reach up to 700 mL per sample (Table 5). The most common eluents in SPE of PBDEs in seafood are hexane or hexane:dichloromethane in different proportions, using diverse SPE sorbents, frequently in series. Furthermore, as previously mentioned, SPE cleanup is preceded by an extractive step, generally CSE or PLE (Table 5), so the overall time and solvent consumption should take that into account. If CSE is used, then there is an even greater quantity of residual lipid to be removed, while ready-to-analyse extracts without any additional clean-up step may be obtained by using fat retainer sorbents inside the PLE cell [61–65]. Finally, after SPE cleanup, there is always a need of a concentration step.

It seems that, regardless of matrix fat content, a single SPE step is enough to yield adequate recovery rates [46,56,57,66,67], although more exhaustive cleanup (up to three sequential SPE) can also be employed, even for low fat samples [58,68]. Additionally, different eluents can be sequentially added for fractionation, thus separating PBDEs from MeO-PBDEs and OH-PBDEs, in order to improve chromatographic resolution [69].

5.3. Integrated extraction and cleanup

The suitability of integrating a cleanup step into SFE or PLE techniques has been achieved by the use of sorbents in the

extraction cell which would trap the undesired matrix components [45,61,62].

QuEChERS is a unique analytical methodology, originally designed for extraction/isolation of polar pesticides from fruits and vegetables [70], whose use has already been extended to other pollutants, including PBDEs. This method involves micro scale extraction using acetonitrile, or other organic solvents, followed by addition of magnesium sulphate alone or with other salts, generally sodium chloride. By varying the magnesium sulphate:sodium chloride ratio, it is possible to refine the polarity range to avoid co-extraction of interfering substances.

Besides requiring reduced sample sizes and small extraction volumes of less toxic solvents (Table 4), it makes use of common laboratory material and encompasses a small number of steps. As a result, potential sources of systematic and random errors are diminished. Notwithstanding, QuEChERS is a very versatile method that allows working at different pHs and several modifications can be performed at the cleanup step, although this technique cannot easily be automated and the enrichment factor is very poor.

While column-based SPE is often associated with CSE and PLE, d-SPE commonly follows QuEChERS extraction (Table 5). d-SPE is a simple and straightforward cleanup method based on the addition of a sorbent or sorbent mixture into the extract in order to remove the matrix interferences, which is then separated by centrifugation. A standard sorbent mixture used in d-SPE may contain PSA (to remove polar interferences, as organic acids, polar pigments and some sugars), GCB (to remove sterols and pigments such as chlorophyll and carotenoids) and C_{18} (to remove non-polar interferences, like lipids) [70].

In relation to SPE, d-SPE requires a negligible or inexistence amount of cleanup solvent, thus being a greener approach (Table 5). Moreover, d-SPE ensures larger and more reproducible recoveries of analytes with acidic or basic properties (as OH-PBDEs). Hence, d-SPE is by far faster, cost-effective and sustainable, since it uses less sorbent and solvent, smaller amounts of sample and less apparatus and consumables. Plus, it ensures a better interaction between the sorbent and the extract for cleanup [70].

MSPD is a simple and versatile method based on SPE, though it starts with the blend of a solid sample with an appropriate sorbent (usually the ones used in SPE) until a homogeneous dry mixture is obtained. This MSPD blend may then be packed into an empty column (with frits in both edges) for fractionation with a suitable organic eluent.

Labadie and colleagues [57] used ultrasound assisted-MSPD with activated silica gel and sulphuric acid-impregnated silica gel, followed by sulphuric acid digestion and multilayer cartridge cleanup to promote complete lipid removal and elimination of matrix effects during instrumental analysis. High recovery rates (greater than 70%) were achieved for all twenty-eight PBDEs, including perbrominated BDEs, though high solvent consumption was necessary (more than 100 mL per 3 g of freeze-dried sample) [57]. Miniaturization of MSPD using C_{18} and Florisil as dispersants and acetonitrile:water as eluting solvent resulted in lower sample size (0.1 g) and low sorbent (0.4 g) and solvent (2.6 mL) request, with satisfactory recoveries (between 55% and 130%) for tetra- and penta-BDEs [71]. Liu and co-workers [72] reported high recoveries (higher than 90%) of PBDEs and MeO-PBDEs using a MSPD method with hexane:dichloromethane (1:1, v/v), hexane:acetone (1:1, v/v) or acetone as eluents. Among all tested eluents, only acetone led to moderate recoveries of OH-PBDEs (near 80%), once strong polar-polar interactions occurred between OH-PBDEs and the sorbents (polar Florisil and CCG) [72]. For extraction and cleanup of all three classes, only 2 mL of total solvent were required. Still, additional sulphuric acid cleanup was required for fish samples. While the amount of CCG used did not affect PBDEs and MeO-PBDEs recoveries, this variable played a major role in extracting OH-PBDEs (preferably CCG:sample, 1:10), possibly due to the polar moieties of CCG that may induce dispersion of polar components of samples and extraction of polar compounds [72].

MSPD is an excellent alternative to conventional methods since it is a simple, time- (around 15 min per sample) and cost-effective technique that requires a small sample size and low solvent demand, plus it does not require the preparation and maintenance of equipment, and offers the possibility of simultaneously performing extraction and cleanup. Yet, MSPD also presents few drawbacks, since it does not allow automation and may require an additional cleanup step.

Taking into account the extensive variability among available analytical protocols, Table 6 highlights the general performance of extraction and cleanup methods commonly used in PBDEs and their metabolites in seafood.

6. Instrumental analysis

Owing to their volatility and polarity, GC is often selected as the analytical separation method for PBDEs and MeO-PBDEs, while LC is commonly chosen for OH-PBDEs analysis. An overview of the main instrumental parameters used in the analysis of PBDEs and their metabolites in seafood studies is presented in Table 7.

6.1. Injection

The injection of sample extracts into the GC system is a determinant step for achieving accurate results, especially when target compounds present high boiling points. Automatic sample injection should be employed whenever possible to improve

Table 6
Overall performance of extraction and cleanup methods commonly used in PBDEs and their metabolites in seafood.

Method		Efficacy	Organic solvent consumption	Time	Cost	Automation	Operational ease
Extraction	CSE	+	−	−	+	−	++
	PLE	+	+	+	−	++	−
	UAE	+	+	+	+	−	−
	MAE	+	+	+	−	+	−
	SFE	+	++	+	−	+	−
Cleanup	GPC	+	−	−	−	+	+
	SPE	++	−	−	+	+	+
Integrated extraction/cleanup	QuEChERS d-SPE	++	++	++	++	−	++
	MSPD	+	+	+	++	−	+

(−), less favourable; (+), favourable; (++), more favourable.
CSE, Conventional Soxhlet Extraction; d-SPE, dispersive-Solid Phase Extraction; GPC, Gel Permeation Chromatography; MAE, Microwave-Assisted Extraction; MSPD, Matrix Solid-Phase Dispersion; PLE, Pressurized Liquid Extraction; SFE, Supercritical Fluid Extraction; SPE, Solid-Phase Extraction; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; UAE, Ultrasound-Assisted Extraction.

Table 7
Instrumental parameters for PBDEs, MeO- and OH-PBDEs analysis.

Sample	Congeners (total)	GC/LC	Injection mode (temperature)	Column system		Detector	Ref
				Stationary phase	Dimensions		
Muscle (bream, carp, gibel carp, roach, rudd, pike, pikeperch, tench, wels, white bream)	Tri- to hepta-BDEs (7)	GC	Cold splitless (−)	HT-8	10 m × 0.10 mm × 0.10 μm	ECNI-MS	[83]
Muscle (barbel, carp)	Tri- to deca-BDEs (11)	GC	Splitless (275°C)	HP-5MS	30 m × 0.25 mm × 0.25 μm	NICI-MS	[65]
Muscle and **liver** (bighead carp, tilapia)	Di- to deca-BDEs (11)	GC	− (290°C)	DB-5MS	30 m × 0.25 mm × 0.25 μm (di- to hepta-BDEs) and 15 m × 0.25 mm × 0.10 μm (deca-BDE)	EI-MS/MS	[95]
Muscle (Nile perch, Nile tilapia)	Tri- to deca-BDEs (11)	GC	Splitless (−)	DB-5MS	30 m × 0.25 mm × 0.10 μm	IT-HRMS	[58]
Muscle and **whole soft tissue** (salmon, prawn, mussels, tuna)	Tri- to deca-BDEs (10)	GC	PTV (90°C)	HT-8	25 m × 0.22 mm × 0.25 μm	EI-MS	[68]
Muscle (tilapia)	Tri- to deca-BDEs (9)	GC	PTV (−)	DB-5MS	30 m × 0.25 mm × 0.25 μm (di- to hepta-BDEs) and 10 m × 0.25 mm × 0.10 μm (deca-BDE)	NICI-HRMS	[76]
Muscle and **whole soft tissue** (mussel, yellow croaker, softshell clam)	MeO-tetra- to MeO-penta-BDEs (9)	GC	Splitless (280°C)	DB-5MS	30 m × 0.25 mm × 0.10 μm	EI-MS	[69]
Plasma (brown bullhead)	OH-di- to OH-penta-BDEs (20), MeO-tri- to MeO-penta-BDEs (20) and tetra- to hepta-BDEs (36)	GC	Split/splitless (280°C)	DB-5MS	60 m × 0.25 mm × 0.25 μm (MeO-PBDEs) and 15 m × 0.25 mm × 0.10 μm (PBDEs)	EI-HRMS	[49]
Whole body (Pacific herring, trout)	OH-tri-BDEs (4), MeO-tetra-BDEs (7) and mono- to deca-BDEs (39)	GC	Splitless (300°C)	DB5-HT (mono- to hepta-BDEs), DB5 (octa- to deca-BDEs, OH-PBDEs and MeO-BDEs)	17 m × 0.25 mm × 0.1 μm (mono- to hepta-BDEs), 5 m × 0.25 mm × 0.1 μm (octa- to deca-BDEs), 30 m × 0.25 mm × 0.25 μm (OH-PBDEs and MeO-BDEs)	HRMS	[86]
Whole soft tissue (ark shell, blue mussel, razor clam, short-necked clam, surf clam)	OH-tri- to OH-hexa-BDEs (5), MeO-tri- to MeO-hexa-BDEs (14)	GC	Splitless (280°C)	DB-5MS	30 m × 0.32 mm × 0.25 μm	ECNI-MS	[88]
Muscle and **whole soft tissue** (mussel, yellow croaker, softshell clam)	OH-tri- to OH-penta-BDEs (5)	LC	−	Dionex C_{18}	100 mm × 2.1 mm × 2.2 μm	Negative ESI-MS/MS	[69]
Whole soft tissue (marine sponge)	Mono- to di-OH-tetra-BDEs (5)	LC	−	Shim-pack FC-ODS	150 mm × 4.6 mm × 3.0 μm	Negative APCI-MS/MS	[79]
Muscle (carp)	OH-di- to OH-tetra-BDEs (8)	LC	−	Xbridge C_{18}	50 mm × 2.1 mm × 3.5 μm	Negative ISP-MS/MS	[51]

APCI, atmospheric pressure chemical ionization; BDE, Brominated diphenyl ether; ECNI, electron-capture chemical ionization; EI, electron impact ionization; ESI, electrospray ionization; GC, gas chromatography; ISP, ion-spray ionization; IT, ion trap; HRMS, high resolution mass spectrometry; LC, liquid chromatography; MS, mass spectrometry; NICI, negative ion chemical ionization; PTV, programmable-temperature vaporizer.

reproducibility and, consequently, overall method's precision. Few injection techniques have been reported concerning PBDEs and MeO-PBDEs extracts, being splitless, pulsed splitless, PTV, and on-column injectors, the most common ones.

Since split/splitless injection requires the use of high injection temperatures (250–300°C), thermal degradation (presence of a hump or an increase in baseline before the peak) of higher brominated BDEs, usually BDE 209, may be observed, thus requiring careful optimization [73].

This thermal degradation and discrimination can also be avoided by the use of on-column injection, since the sample extract is introduced directly into the column [73,74]. Nevertheless, this technique demands a more careful extract cleanup than split/splitless injection to prevent instrumental problems, as increased noise or fast column deterioration.

Since PTV inlets allows a higher injection volume (up to 125 μL) [75], it may help to achieve good LODs, thus this technique is becoming more and more popular in the analysis of PBDEs and their metabolites in seafood samples [68,76,77]. The use of PTV requires a previous optimization (e.g. injection rate, injection temperature, vent flow, temperature rate, transfer temperature, transfer time, injection volume and solvent elimination time) [75] and it may be operated in different modes. For instance, the "temperature programmed pulsed splitless" mode resulted in an overall increased yield of BDE congeners, mainly of highly brominated ones, which present lower vapour pressure [73].

Furthermore, the cleanliness of the liner should not be disregarded, since active sites on dirty liners can also lead to adsorption effects and thermal degradation of target compounds. Such adsorption may be prevented by adding 0.1–0.35% (v/v) of dodecane to the sample solution with multibaffled liners to trap the low boiling congeners, as well as changing the liner every 100–200 injections [75].

6.2. Column system

Highly brominated PBDEs may also suffer degradation at retention gaps. According to Björklund and colleagues [73], Siltek® deactivated retention gaps may minimize such degradation, since they are not as susceptible to the formation of active silanols as other retention gap deactivation techniques.

The column length and film thickness are two relevant features that affect the retention time of a certain analyte. If a target compound presents low volatility, owing to its high molecular weight (e.g. hepta-to deca-BDEs), then short GC columns (10–15 m) with lower film thickness (less than 0.25 μm) should be employed to reduce residence time and avoid excessive elution temperatures and possible thermal degradation [28,73,78]. This fact explains why the determination of BDE-209 is often done separately, using stationary phases of a lower thickness (0.1 μm) and/or a shorter column, thus improving its detection (Table 6). Still, columns of 25–50 m length and 0.1–0.3 μm thickness are preferable to achieve better peak resolution for all PBDEs and metabolites (Table 6). Even so, BDE-33 co-elution with BDE-28 remains very usual [78].

Mainly non-polar columns, as DB-5MS/HP-5MS, STX-500 and DB-XLB, are used in the chromatographic separation of PBDEs and metabolites (Table 6). However, the latter was found to lead to a complete degradation of BDE-209, while DB-1 showed a better performance [78]. Finally, it is worth mentioning that helium is the reference carrier gas for such analyses.

When OH-PBDEs are separated by LC methods, C_{18} reverse-phase columns (50–150 mm) are employed (Table 6), being subjected to either isocratic elution or eluent gradient, both consisting of mixtures of water, ammonium acetate, methanol and acetonitrile [51,69,72,79].

6.3. Detection

6.3.1. Ionization modes

Nowadays, PBDEs and MeO-PBDEs are usually analysed by GC-MS or GC-MS/MS, ion trap or triple quadrupole. Despite recent studies still use GC-ECD [80,81], it is falling into disuse, owing to its limited linear range and lack of selectivity. If GC-ECD is used, the cleanup or chromatographic column will have to separate all other halogenated organic compounds (e.g. PCBs) that may create co-elution problems [82].

GC systems using the ECNI have been the most commonly used for PBDEs and MeO-PBDEs detection in seafood samples [68,83,84]. Quantification is usually accomplished using the SIM mode by monitoring the bromide ion isotopes (m/z 79 and m/z 81), using the GC-MS chiefly as bromine-selective detector. While ECNI offers better sensitivity to higher brominated PBDEs, EI (also named Electron Impact) displays better sensitivity to lower brominated PBDEs (less than four bromines) [28]. Moreover, isotopically labelled standards (^{13}C) cannot be used as internal standards in ECNI mode if only the bromide ions are being monitored. Nevertheless, mono fluorinated BDEs are a good alternative when co-elution with sample PBDEs does not occur. On the contrary, EI allows the use of ^{13}C labelled internal standards.

Mackintosh and co-workers evaluated the analytical performance of a triple quadrupole mass spectrometer compared to a HR mass spectrometer for the analysis of PBDEs in fish samples [85]. They have verified that IDLs for the GC-MS-MS ranged from 0.04 pg/μL/to 41 pg/μL, whereas those for the GC-HR-MS ranged from 5 pg/μL to 85 pg/μL. IDLs for MeO-PBDEs and OH-PBDEs may differ in three orders of magnitude, depending on congeners under study, reaching only a few tens of picogram injected into the GC column [86,87].

Despite being increasingly unusual, OH-PBDEs may also be analysed by GC-MS, being subjected to derivatization (e.g. diazomethane or pentafluorobenzoyl chloride/tetra-n-butylammonium hydroxide as derivatizing agents) prior to injection, resulting in the corresponding MeO-PBDEs [49,88]. By including a derivatization step, there is an increase of the overall sample analysis time and a possible yield reduction, plus many of such methylating derivatizers are considerably toxic [51]. Hence, LC methods for analysing OH-PBDEs are becoming more popular. Nevertheless, in order to determine derivatized OH-PBDEs by GC-MS, EI should be favoured over ECNI, since the lack of specific masses other than bromine ions (m/z 79 and m/z 81) hampers identification.

Nowadays, LC–MS/MS is a major analytical alternative for the analysis of OH-PBDEs. A number of different ionization modes have been applied in seafood studies, mostly ESI, but also IS and APCI all operating in negative mode [51,69,72,79,89]. The IDLs of OH-PBDEs using the aforementioned ionization techniques in LC–MS varied between 0.01 and 9.1 pg/μL [51,69,79].

6.3.2. Fragmentation patterns

As revised by Hites [90], the major fragments formed in EI are the molecular ions that can be used for identification and quantification purposes, followed by other fragment ions (mostly single, but also doubly charged ions due to the loss of Br_2 from the molecular ion), often used for confirmation. Hence, selected ion monitoring of the $(M\text{-}Br_2)^+$ ion is advisable for PBDEs determination. However, BDE-77 presents a singular mass spectrum, since it shows almost no $(M\text{-}Br_2)^+$ ion [90].

On the other hand, ECNI spectra of PBDEs with seven or less bromines are mainly represented by Br^- and HBr^{2-} (in a lesser extent), while higher brominated BDEs also reveal tetra- or pentabromophenoxide ions, due to cleavage of the phenyl-ether linkage, since the negative charge is likely located on the oxygen atom

[90]. Therefore, m/z 79 and 81 are preferred for PBDEs with seven or less bromides, while phenoxide ions are analytically useful for quantitation of the octa- to deca-BDEs (excepting BDE-206 whose spectra present high intensities of $(M\text{-}Br_3)^-$ and $(M\text{-}Br_4)^-$) [90]. An analytical method may also take profit of ion source temperature impact on the abundance of Br^- in the ECNI spectra, since higher temperatures increase sensitivity [90].

As regards to MeO-PBDEs, ECNI spectra are also dominated by Br^- and HBr_2^-, which as already mentioned before, hinders compound identification. Still, the abundance of HBr_2^- ions are slightly higher for *meta*- and *para*-substituted, while *ortho*-substituted MeO-PBDEs have a variable abundance of m/z 186/8 and 266 [90].

Contrariwise, EI spectra allow the distinction between *ortho*, *meta* or *para* position of methoxyl in relation to the phenyl-ether linkage. Albeit $(M\text{-}Br_2)^+$ ion is prominent in PBDE spectra, for MeO-PBDEs such is only verified for *meta*-substituted ones, which also significantly produce $(M\text{-}CH_3Br_2)^+$ ions [90]. Concerning *ortho*-substituted congeners, the $M\text{-}CH_3Br$ ion is highly abundant, likely due to the production of a stable brominated dibenzo-*para*-dioxin ion [90]. As for *para*-substituted congeners, $M\text{-}CH_3$ ions are consistently present [90].

For non-derivatized OH-PBDEs, ionized by IS operating in negative mode, different mass spectra were observed according to the position of hydroxyl in relation to the phenyl-ether bond [51]. For instance, for *ortho*-hydroxylated BDEs only the formation of Br^- was verified. On the contrary, *meta*-substituted OH-PBDEs suffered bromine abstraction, cleavage of the ether bond producing bromophenoxide ions and resulted in the formation of both Br^- isotopes. Finally, *para*-substituted OH-PBDEs mass spectra represented the formation of bromobenzoquinone anions and both Br^- isotopes.

Few authors have performed quantitative analysis of OH-PBDEs by LC-ESI-MS [69,72]. For *ortho*-substituted OH-PBDEs, only m/z 79 and m/z 81 were used for quantification purposes, while for *meta*-hydroxylated BDEs, Br^- and $(M\text{-}Br)^-$ ions were favoured. Concerning *para*-substituted OH-PBDEs, a loss of m/z 265 occurred (likely corresponding to $[C_6H_2Br_2O_2]^-$), that was selected for quantification.

7. Quality assurance/Quality control

7.1. Quality assurance

The sample under study may suffer contamination throughout the analytical procedure, mostly by solvents and reagents used, but it can also occur even before the sample arrives to the laboratory, i.e. during sampling, sample handling and pre-treatment. Such can happen owing to the environment, packing materials or instruments used during sample preparation. It shall not be forgotten that PBDEs have a widespread existence, so they are very likely present in materials and equipment used for sample handling and treatment.

No plastic materials, except polyethylene or polytetrafluorethene, should be used for sampling, due to the possible exchange of contaminants from or into the container material [28]. Seafood dissection should be performed in a clean bench, always avoiding contact with potentially PBDE-containing materials and products [28]. It should be undertaken by trained personnel wearing clean gloves, and using clean stainless steel knives, scalpels, and homogenizers [28]. Afterwards, the working bench, all tools and equipment used for sample preparation should be washed with standard laboratory detergent, as Decon® 90, and rinsed thoroughly with deionized water followed by a solvent [28].

Finally, processed samples should be stored in clear and indelible labelled solvent-washed aluminium cans or glass jars, preferably prebaked at 450°C [28].

7.2. Quality control

Nowadays, there any many companies offering high-purity calibration and internal standards (^{13}C, ^{2}H or fluorinated analogues) for PBDEs and MeO-PBDEs, but the same does not apply for OH-PBDEs. Still, isotope dilution analysis is particularly recommended in trace determinations since intermediate manipulations of the sample (from extraction to chromatographic separation) as well as any changes in instrumental sensitivity will not affect the final result as any aliquot of the isotope diluted sample will show the same isotope composition [91].

Howsoever, a multilevel calibration curve with at least five points should be employed for quantification. Calibration standards prepared from high-purity standard solutions should be stored in the dark and preferably in amber glass gas tight flasks, to prevent photodegradation and solvent evaporation. Also, the use of adequate internal standards as recovery and syringe standards is advisable.

A procedural blank should also be prepared to help defining the method and instrumental limits of detection and quantification. According to Webster and colleagues [28], achievable limits of determination based on a 10 g WW biota sample for each individual component, are as follows: 1) GC-ECNI-MS: 0.05 ng/g for tri- to hepta-BDEs and 0.50 ng/g for BDE-209; 2) GC-EI-MS: 0.5 ng/g; and 3) High-resolution GC-MS: 0.02 pg/g for tri- to hepta-BDEs and 0.5 pg/g for BDE-209.

The analyst should also evaluate the method's precision and repeatability. The accuracy of an analytical method should fall between 70% and 120% recovery rate, and it may be evaluated either by sample fortification, LRM or CRM analysis [28]. Both LRM and CRM should be of the same matrix type as the samples under study. Few CRMs certified for PBDEs in seafood are available at the NIST, as "SRM 1947 – Lake Michigan Fish Tissue".

From time to time, it is recommended that the laboratory participate in interlaboratory exercises, as those from the QUASIMEME programme, aiming to provide an independent evaluation on a certain analytical method's performance.

8. Conclusions

PBDEs constitute a relevant class of additive BFRs from an economical and environmental perspective, mainly due to its proven toxicity and bioaccumulation ability. Likely, as an attempt to provide a solid knowledge on their occurrence in seafood (the major exposure pathway for humans), to enable these contaminants to become duly regulated, a multiplicity of research initiatives has focused such assessment. For this purpose, alternative extraction techniques, such as PLE or SFE, have been developed to replace lingering CSE. These new extraction techniques offer the advantages of significantly reducing the organic solvent consumption, favouring automation and allowing the introduction of an on-line cleanup step. However, due to their high investment costs, CSE still remains the most widely used technique for PBDEs extraction from seafood samples.

In general, extraction techniques are followed by GPC and/or SPE for purification and fractionation of extracts, which undoubtedly provide good analyte recoveries, but often require high extraction volumes. Emerging techniques such QuEChERS or UAE, followed by d-SPE, have only been employed in a limited number of studies so far. Nevertheless, their promising results show that their application may likely expand in the near future. Encouraging the use of sustainable analytical methods not only reduces waste generation in the laboratory, but it also leads to a global reduction in environmental pressure with subsequent health improvements.

Only with adequate analytical tools and data from wide and regular monitoring surveys will enable to provide the necessary information for implementing effective food safety regulation.

Notes

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